Feed Restriction Significantly Alters Lipogenic Gene Expression in Broiler Breeder Chickens[1]

Mark P. Richards,[2] Stephen M. Poch, Craig N. Coon,* Robert W. Rosebrough, Christopher M. Ashwell and John P. McMurtry

*U.S. Department of Agriculture, ARS, Growth Biology Laboratory, Beltsville, MD 20705-2350 and *University of Arkansas, Center of Excellence for Poultry Science, Fayetteville, AR 72701*

ABSTRACT Broiler breeder pullets were divided into two groups at 21 wk of age. One group was given free access to feed (ad libitum) and the other fed a limited amount of feed (restricted). At 22 wk, all birds were photostimulated and maintained throughout an egg-laying cycle ending at 36 wk. Samples of liver and abdominal fat pad were collected just before photostimulation (prelight), after photostimulation at first egg and at peak egg production (plateau). Hepatic expression of sterol regulatory element binding protein-1, ATP-citrate lyase, fatty acid synthase, malic enzyme, acetyl-CoA carboxylase and stearoyl-CoA (Δ9) desaturase 1 genes in ad libitum birds declined from their highest levels just before photostimulation as the birds came into and maintained egg production. In contrast, the restricted birds had significant ($P < 0.05$) increases in the expression of these genes after photostimulation at first egg with a subsequent decline as they reached peak egg production. Hepatic expression of fatty acid binding protein, VLDL apolipoprotein (apoVLDL-II) and apoB genes increased significantly ($P < 0.05$) in both ad libitum and restricted breeders after photostimulation, whereas apoA1 gene expression declined during this time. Abdominal fat pad weights were significantly ($P < 0.05$) higher in the ad libitum compared with restricted birds after photostimulation. Lipoprotein lipase in this tissue showed a pattern of expression similar to that observed for the hepatic lipogenic enzyme genes. In conclusion, feed restriction during the pullet-to-breeder transition period significantly ($P < 0.05$) altered hepatic lipogenic gene expression in broiler breeders. J. Nutr. 133: 707–715, 2003.

KEY WORDS: • *gene expression* • *lipid metabolism* • *lipogenesis* • *mRNA* • *chickens*

The modern commercial broiler is the product of intensive selection over many generations for rapid growth and enhanced muscle mass. Selection for these economically important traits has been accompanied by an increase in voluntary feed intake, resulting in birds that do not adequately regulate feed intake to achieve energy balance (1). Thus, broiler chickens are prone to obesity resulting from hyperphagia when given free access to feed. Although unrestricted feeding of these birds with typical starter and breeder rations would ensure an adequate nutrient supply to support growth and development, most commercial broiler feeding programs utilize varying levels of feed restriction as a management tool to regulate body weight, improve egg production and promote flock uniformity during the rearing and breeding phases of production (2,3). There have been many reports comparing ad libitum vs. restricted feed intake and changes in feed composition on various aspects of welfare, growth, body composition and egg production in broiler breeders (4–8). Because egg production causes major changes in the metabolism of lipids by breeder hens to meet the demands of yolk formation, it is important to understand the underlying genetic mechanisms governing lipid metabolism, specifically as they are affected by nutritional status.

Birds have the ability to store large quantities of excess energy (in the form of triglycerides) in liver, adipose tissue and in yolk of developing oocytes (9). Lipogenesis (i.e., the conversion of glucose to triglycerides) takes place primarily in the liver of birds (10) and involves a series of linked, enzyme-catalyzed reactions including glycolysis, the citric acid cycle and fatty acid synthesis (**Fig. 1**). Hepatic lipogenesis is subject to both nutritional and hormonal control and this metabolic process is highly responsive to changes in the diet (11,12). Adipose tissue serves primarily as a storage site for lipid with little lipogenesis occurring in this tissue (9). Differential lipogenic capacity of liver vs. adipose tissue in birds is a function of the expression of a key transcription factor, sterol regulatory element binding protein-1 (SREBP-1).[3] The gene for SREBP-1 is highly expressed in the liver, but to a much lesser

[1] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by USDA and does not imply its approval to the exclusion of other suitable products.

[2] To whom correspondence should be addressed.
E-mail: richards@anri.barc.usda.gov.

[3] Abbreviations used: ACC, acetyl-CoA carboxylase; ACL, ATP citrate lyase; apo, apolipoprotein; CE/LIF, capillary electrophoresis with laser-induced fluorescence detection; FABP, fatty acid binding protein (liver); FAS, fatty acid synthase; ICDH, isocitrate dehydrogenase; LR, leptin receptor; LPL, lipoprotein lipase (adipose); ME, malic enzyme; MT, metallothionein; RT-PCR, reverse transcription polymerase chain reaction; SCD1, stearoyl-CoA (Δ9) desaturase 1; SREBP-1, sterol regulatory element binding protein-1; T_3, triiodothyronine; VLDLy, yolk-targeted VLDL.

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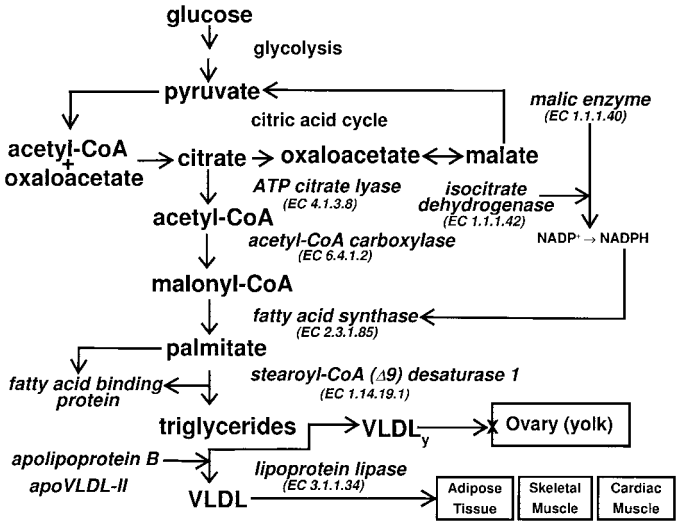

FIGURE 1 Hepatic lipogenesis and export of lipid depicting some of the reactions involved in the conversion of glucose to triglyceride. Key lipogenic enzymes are listed, as well as lipid binding and transport proteins involved in the intracellular transport of fatty acids and the export of triglycerides from the liver to other tissues such as adipose tissue and muscle. Lipid uptake by adipose and muscle tissue, but not oocytes, requires the action of lipoprotein lipase. Lipid uptake by oocytes involves a specific yolk-targeted VLDL particle termed $VLDL_y$.

extent in adipose tissue (13). Moreover, the expression of a number of lipogenic enzyme genes such as fatty acid synthase (FAS), malic enzyme (ME), acetyl CoA carboxylase (ACC), ATP citrate lyase (ACL) and steroyl CoA (Δ9) desaturase 1 (SCD1) is directly influenced by SREBP-1 (12–14). Lipid accumulation by adipose tissue and the developing oocyte depends on plasma lipid that is derived from hepatic lipogenesis and lipid absorbed from the diet. In fact, it has been estimated that 80–85% of the fatty acids present in broiler adipose tissue triglyceride stores were derived from hepatic lipogenesis or from the diet via intestinal absorption (15). Therefore, hepatic lipogenesis and the export of lipid are crucial steps linked to adipose tissue lipid accretion, as well as to oocyte growth and maturation (i.e., yolk formation) in egg-laying hens. The nutritional state of the bird, as determined by the amount and composition of feed consumed, dramatically affects hepatic lipid metabolism (11). Variations in nutrient intake and status are communicated to the liver and other internal organs by alterations in the plasma levels of hormones [e.g., insulin, glucagon, triiodothyronine (T_3)] and metabolites (e.g., glucose, free fatty acids) that respond acutely to dietary changes. To determine the metabolic consequences of feed restriction on body composition and egg production in broiler breeder pullets, we investigated changes in the levels of key metabolic hormones and the expression of selected hepatic lipogenic genes during the pullet-to-breeder transition period.

MATERIALS AND METHODS

Stock and management. Cobb 500 broiler breeder pullets were fed a controlled amount of feed to maintain optimum body weight according to Cobb Breeder Management Guide specifications (16) until they reached 21 wk of age. At this time, the birds were placed in individual laying cages and half were switched to unrestricted feeding (ad libitum) with Breeder I feed (11.82 MJ/kg metabolizable energy; 16 g crude protein/100 g feed); the remaining birds were fed a restricted amount of the same ration according to Cobb guidelines (restricted, see **Table 1**). At 22 wk, all birds were photostimulated and maintained throughout a laying cycle ending at 36 wk. Photostimulation consisted of changing the photoperiod from 8 h light:16 h dark to 12 h light:12 h dark. Body weights were determined and samples of plasma, liver and abdominal fat pad were collected at the following times: *1*) 1 d before photostimulation (prelight) at 22 wk, *2*) at first egg (~24 wk) and 3) through peak egg production (plateau, 36 wk). Total egg production was monitored through 36 wk for some birds (plateau groups). Tissue samples were snap frozen in liquid nitrogen at the time of their collection and stored at −80°C before analysis. Plasma was stored frozen (−20°C) before analysis. All procedures followed established protocols approved by institutional animal care and use committees.

Hormone and enzyme assays. Specific immunoassay techniques were used to determine plasma levels of insulin (17), glucagon (kit, Linco Research, St. Charles, MO), T_3 (18) and 17β-estradiol (kit, Diagnostics Products, Los Angeles, CA). Plasma samples were treated according to kit protocols or as described previously (17,18). Cytosolic ME (EC 1.1.1.40) activity in liver tissue samples was determined and expressed as μmol of oxidized or reduced NADP/(min · g liver) at 30°C as described previously (19).

Gene expression analyses. Total RNA was isolated using the TRIzol reagent according to the manufacturer's protocol (Invitrogen/Life Technologies, Carlsbad, CA). RNA integrity was assessed via agarose gel electrophoresis and RNA concentration and purity were determined spectrophotometrically using A_{260} and A_{280} measurements. Reverse transcription (RT) reactions (20 μL) consisted of 1 μg total RNA, 50 U SuperScript II reverse transcriptase (Invitrogen/Life Technologies), 40 U of an RNAse inhibitor (Invitrogen/Life Technologies), 0.5 mmol/L dNTP, and 100 ng random hexamer primers. Polymerase chain reaction (PCR) was performed in 25 μL containing 20 mmol/L Tris-HCl, pH 8.4, 50 mmol/L KCl, 1.0 μL of the RT reaction, 1.0 U of Platinum Taq DNA polymerase (Hot Start, Invitrogen/Life Technologies), 0.2 mmol/L dNTP, 2.0 mmol/L Mg^{2+} (Invitrogen/Life Technologies), 10 pmol each of the gene specific primers and 10 pmol each of the primers specific for β-actin (see **Table 2** for primer sequence). Thermal cycling parameters were as

TABLE 1

Feed allocation program for restricted feeding of broiler breeder chickens during egg production

Week	Feed allotment[1]	Notes[2]
	$g \cdot bird^{-1} \cdot d^{-1}$	
21	113	
22	118	Photo-stimulation
23	127	
24	132	
25	139	5% egg production, 1.64 MJ/d ME
26	151	
27	160	
28	165	60% egg production, peak feed, 1.96 MJ/d ME
29	165	
30	165	Peak egg production
31	164	
32	163	
33	162	
34	161	
35	160	
36	159	

[1] Based on recommendations of the Cobb 500 Breeder Management Guide (16).

[2] Specific production/management milestones are listed such as when the restricted birds were photo-stimulated (wk 22), when the birds achieved 5%, 60% and peak egg production (wks 25, 28 and 30, respectively), when the maximum amount of feed was provided (wk 28), and energy (metabolizable energy, ME) intake of the restricted birds at the beginning of egg production and at maximum (peak) feed allocation (wks 25 and 28, respectively).

TABLE 2

Oligonucleotide PCR primers

Gene[1]	Accession number[2]	Primer sequence (5′→3′)	Orientation	Product size (bp)
1. β-Actin	L08165	TGCGTGACATCAAGGAGAAG	Forward	300
		TGCCAGGGTACATTGTGGTA	Reverse	
2. SREBP-1	AY029224	GAGGAAGGCCATCGAGTACA	Forward	392
		GGAAGACAAAGGCACAGAGG	Reverse	
3. ME	AF408407	ATGAAGAGGGGCTACGAGGT	Forward	470
		CCCATTCCATAACAGCCAAG	Reverse	
4. ACL	AJ245664	GGTGACCACAGGCAGAAGTT	Forward	452
		ACCCCTTCATAGACCCCATC	Reverse	
5. ACC	J03541	CACTTCGAGGCGAAAAACTC	Forward	447
		GGAGCAAATCCATGACCACT	Reverse	
6. FAS	J04485	GGAGTCAAACTAGTTATCCATGGCC	Forward	423
		AAAGGAGATTCCAGCATCGTGCAGC	Reverse	
7. SCD1	X60465	TCCCTTCTGCAAAGATCCAG	Forward	402
		AGCACAGCAACACCACTGAG	Reverse	
8. FABP	AF380999	GAGCTCCAGTCCCATGAAAA	Forward	202
		TCAGCAGCTCCATCTCACAC	Reverse	
9. ApoB	M18421	CACGCCTCACAGACCAAGTA	Forward	407
		CCAGTCAAACGGCACATCTA	Reverse	
10. ApoVLDL-II	M25774	ATGGTGCAATACAGGGCATT	Forward	196
		GGGAAACATCCAGCAAGAAC	Reverse	
11. ApoA1	M18746	AGATGTGGCTGAAGGACACC	Forward	403
		ATCTTCTCACGCAGGTTGCT	Reverse	
12. LPL	X14670	GGTAGACCAGCCATTCCTGA	Forward	401
		AAGCAGACTCGTGTGCAGAA	Reverse	
13. ICDH	EST[3]	CCCAGTTTGAAGCCAAGAAG	Forward	202
		TCAACAGTCTTGCCATCAGG	Reverse	
14. MT	X06749	CCTCAGGACTGCACTTGTGC	Forward	182
		TGGCAGCAGCTGCACTTGCT	Reverse	
15. LR	AB033383	CAGTGTGAGCCGGTACGTTA	Forward	603
		GGAACATCTTCCCAGAGCAG	Reverse	

[1] ACC = Acetyl-CoA Carboxylase; ACL = ATP Citrate Lyase; ApoA1 = Apolipoprotein A1; ApoB = Apolipoprotein B; ApoVLDL-II = Very Low Density Apolipoprotein II; FABP = Fatty Acid Binding Protein (Liver); FAS = Fatty Acid Synthase; ICDH = Isocitrate Dehydrogenase; LR = Leptin Receptor; LPL = Lipoprotein Lipase (Adipose); ME = Malic Enzyme; MT = Metallothionein; SCD1 = Stearoyl-CoA (Δ9) Desaturase 1; SREBP-1 = Sterol Regulatory Element Binding Protein-1.

[2] GenBank accession number.

[3] Designed from expressed sequence tag.

follows: 1 cycle 94°C for 2 min, followed by 30–35 cycles, 94°C for 30 s, 58–60°C for 30 s, 72°C for 1 min with a final extension at 72°C for 8 min.

Capillary electrophoresis with laser-induced fluorescence detection (CE/LIF). Aliquots (2 μL) of RT-PCR samples were diluted 1:100 with deionized water before CE/LIF. A detailed description and validation of the CE/LIF technique used in this study for quantitative analysis of gene expression was reported previously (20). Briefly, a P/ACE MDQ CE instrument (Beckman Coulter, Fullerton, CA) equipped with an argon ion LIF detector was used. Capillaries were 75 μm i.d. × 32 cm μSIL-DNA (Agilent Technologies, Folsom, CA). EnhanCE dye (Beckman Coulter,) was added to the DNA separation buffer (Sigma, St. Louis, MO) to a final concentration of 0.5 g/L. Samples were loaded by electrokinetic injection at 3.5 kV for 5 s and run in reverse polarity at 8.1 kV for 5 min. Integrated peak area for the PCR products separated by CE was calculated using P/ACE MDQ software (Beckman Coulter,).

Quantitation of gene expression. The level of gene expression was determined as the ratio of integrated peak area for each individual gene PCR product relative to that of the coamplified β-actin internal standard (20). Values are presented as the mean ± SEM of 5 individual determinations.

Statistical analysis. Data were analyzed by two-way ANOVA using the general linear models (GLM) procedure of SAS software (SAS Institute, Cary, NC). Significant differences among individual group means were determined with Duncan's multiple range test option of the GLM procedure of SAS software. Pearson correlation coefficients for the interrelationship of selected variables were determined using the PROC CORR procedure of SAS software. Significance was set at $P < 0.05$. Linear regression analysis was used to relate changes in gene expression values (peak area ratios) with enzyme activity measurements for ME.

RESULTS

Feed restriction during egg production resulted in significantly ($P < 0.05$) lower body and abdominal fat pad weights compared with unrestricted feeding (**Table 3**). Also, egg production was higher with a significantly ($P < 0.05$) lower incidence of abnormal eggs in the restricted compared with the ad libitum birds (Table 3). Feed restriction produced significant ($P < 0.05$) effects on circulating levels of key metabolic hormones before the onset of egg production (**Table 4**). Pullets that had been fed a restricted amount of feed for 21 wk before being switched to an ad libitum feed intake exhibited dramatic changes in the levels of insulin, glucagon and T_3. Circulating insulin and T_3 levels were significantly ($P < 0.05$) higher and glucagon levels were significantly ($P < 0.05$) lower in the ad libitum compared with the restricted birds before photostimulation (prelight). Before photostimulation, the molar ratio of insulin to glucagon was 20.20 ± 2.85 vs. 1.44 ± 0.11 for the ad libitum and restricted birds, respectively. When the birds began to lay eggs, this ratio increased significantly ($P < 0.05$) in the restricted group to 9.72 ± 1.11,

TABLE 3

Effects of restricted versus ab libitum feed intake on body and abdominal fat pad weights and egg production for broiler breeder chickens at three stages of egg prodution[1]

	Pre-light		First egg		Plateau	
Variable	Restricted	Ad libitum	Restricted	Ad libitum	Restricted	Ad libitum
Body Weight, *g*	2149 ± 88^{d}	2550 ± 73^{cd}	3042 ± 293^{c}	4097 ± 94^{b}	3742 ± 139^{b}	5045 ± 286^{a}
Fat Pad, *g*	12.5 ± 4.0^{c}	17.5 ± 1.0^{c}	54.5 ± 9.8^{c}	114.6 ± 10.1^{b}	119.8 ± 18.8^{b}	219.4 ± 36.2^{a}
Fat Pad, *g/100 g body*	0.57 ± 0.17^{d}	0.69 ± 0.05^{d}	1.76 ± 0.20^{cd}	2.92 ± 0.24^{bc}	3.24 ± 0.54^{ab}	4.37 ± 0.72^{a}
Total Eggs, *n*	—	—	—	—	57 ± 4	44 ± 7
Abnormal Eggs,[2] %	—	—	—	—	2.04 ± 0.70^{b}	12.44 ± 2.92^{a}

[1] All values are mean ± SEM, $n = 5$. Means within rows with different letter superscripts are significantly different ($P < 0.05$).
[2] Includes soft shell or shell-less, multiple eggs, and multiple soft shell eggs.

whereas it declined significantly ($P < 0.05$) in the ad libitum group to 6.99 ± 1.02 during this time. As egg production progressed to plateau, both groups exhibited declines in this ratio (Table 4).

Limiting feed intake during egg production produced significant ($P < 0.05$) effects on the expression of hepatic genes involved in lipid metabolism, particularly those genes regulating lipogenesis including ME (**Fig. 2***B*), ACL (Fig. 2*C*), ACC (Fig. 2*D*), FAS (Fig. 2*E*) and SCD1 (Fig. 2*F*). In addition, expression of the transcription factor SREBP-1 was influenced in a similar manner by feed restriction (Fig. 2*A*). For the ad libitum group in general, expression of each of these genes relative to β-actin was highest before the onset of egg production (prelight) and declined as the birds came into and maintained egg production. The feed-restricted breeders exhibited significantly ($P < 0.05$) lower lipogenic gene expression levels before photostimulation compared to their ad libitum counterparts. In the restricted group, peak expression levels were delayed until after photostimulation through the onset of egg production (first egg). This was followed by a rapid decline during the plateau phase of the laying cycle.

Hepatic expression levels of apolipoprotein (apo)VLDL-II, apoB and fatty acid binding protein (FABP), genes that code for proteins involved in lipid transport, were also determined (**Fig. 3***A–C*). On the basis of expression profiles in both the ad libitum and restricted birds, it was clear that each of these genes was regulated differently from those involved in lipogenesis. This most likely reflects the involvement of estrogen in the regulation of gene transcription. In general, expression levels relative to β-actin increased significantly ($P < 0.05$) for both ad libitum and restricted groups as the birds came into egg production and remained significantly ($P < 0.05$) elevated during the laying cycle compared with prelight levels. The exact opposite was observed for the apoA1 gene. Expression levels declined throughout the period of egg production in both groups (Fig. 3*D*).

In contrast to the lipid transport genes, lipoprotein lipase (LPL) gene expression in adipose tissue exhibited a pattern similar to the lipogenic enzyme genes (**Fig. 4***A*). This could indicate some coordination between genes regulating lipid production in liver and LPL that mediates lipid uptake by extrahepatic tissues. Feed restriction during early egg production significantly ($P < 0.05$) increased metallothionein (MT) gene expression over that of ad libitum birds before photostimulation, and again at the plateau phase of egg production (Fig. 4*B*). Expression of the isocitrate dehydrogenase (ICDH) gene in liver followed a pattern similar to that observed for MT (Fig. 4C). Both MT and ICDH gene expression profiles were opposite to those observed for the lipogenic enzyme genes (Fig. 2). The up-regulation of hepatic MT and ICDH genes was positively correlated with elevated circulating glucagon levels ($r^2 = 0.87$ and 0.86, respectively) and may signal the presence of stress in the feed-restricted birds. No significant ($P > 0.05$) effects were noted for the expression of the leptin receptor (LR) gene, although there was a trend in both groups toward an increased expression throughout egg production (Fig. 4*D*). Although the differences among groups at each of the experimental sampling periods were small and not significant ($P > 0.05$), this trend indicated a possible role for estrogen in the regulation of LR gene expression.

Significant ($P < 0.05$) correlations were found among the expression levels of various lipogenic genes (**Table 5**). Some of the highest correlations were found among genes encoding hepatic enzymes linked in the lipogenic pathway such as FAS,

TABLE 4

Effects of restricted versus ad libitum feed intake on plasma hormone levels for broiler breeder chickens at three stages of egg production[1]

	Pre-light		First egg		Plateau	
Hormone	Restricted	Ad libitum	Restricted	Ad libitum	Restricted	Ad libitum
Insulin, *nmol/L*	0.303 ± 0.021^{c}	0.609 ± 0.081^{a}	0.535 ± 0.061^{ab}	0.398 ± 0.059^{bc}	0.358 ± 0.014^{c}	0.382 ± 0.019^{c}
Glucagon, *nmol/L*	0.212 ± 0.008^{a}	0.030 ± 0.0004^{e}	0.055 ± 0.001^{d}	0.057 ± 0.003^{d}	0.168 ± 0.003^{b}	0.098 ± 0.007^{c}
Insulin/Glucagon	1.44 ± 0.11^{d}	20.20 ± 2.85^{a}	9.72 ± 1.11^{b}	6.99 ± 1.02^{bc}	2.14 ± 0.08^{d}	4.01 ± 0.38^{cd}
T_3, *nmol/L*	0.37 ± 0.06^{b}	2.27 ± 0.36^{a}	1.11 ± 0.21^{b}	1.20 ± 0.40^{b}	0.68 ± 0.16^{b}	0.94 ± 0.22^{b}
17β-Estradiol, *nmol/L*	0.33 ± 0.08^{c}	0.71 ± 0.12^{bc}	1.25 ± 0.18^{ab}	1.42 ± 0.33^{a}	0.65 ± 0.12^{c}	0.69 ± 0.18^{bc}

[1] All values are mean ± SEM, $n = 5$. Means within rows with different letter superscripts are significantly different ($P < 0.05$).

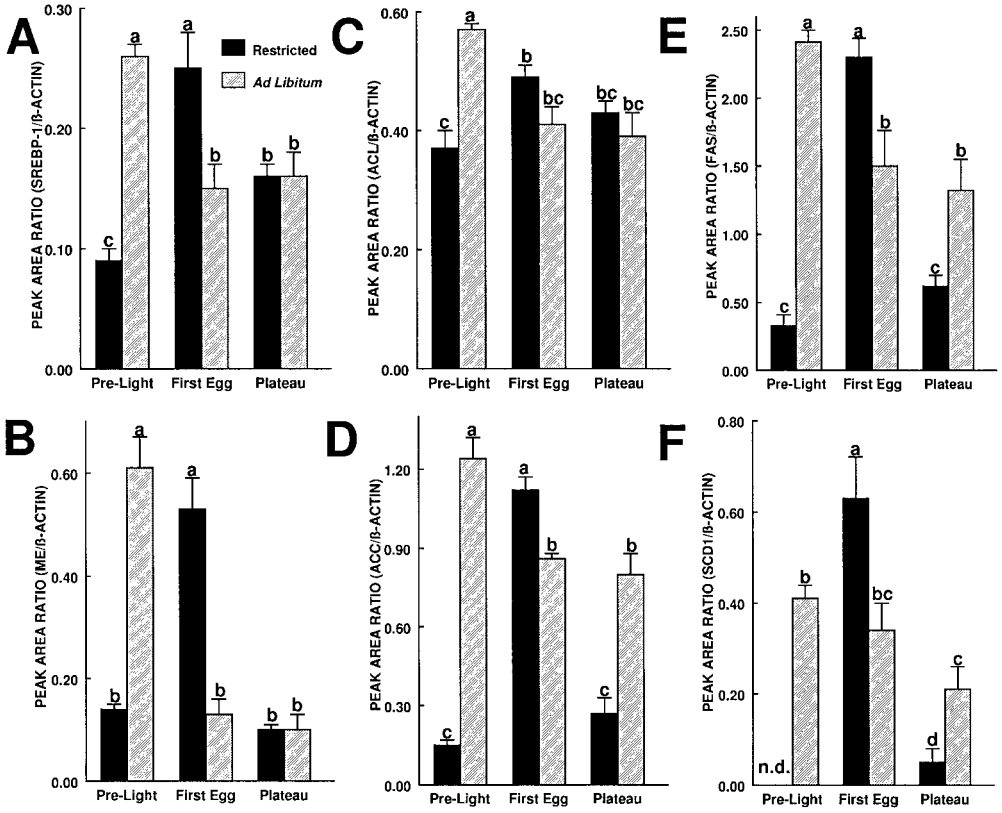

FIGURE 2 Hepatic expression (relative to β-actin) of the lipogenic genes coding for (*A*) sterol regulatory element binding protein-1 (SREBP-1), (*B*) cytosolic malic enzyme (ME), (*C*) ATP citrate lyase (ACL), (*D*) acetyl-CoA carboxylase (ACC), (*E*) fatty acid synthase (FAS) and (*F*) stearoyl-CoA (Δ9) desaturase 1 (SCD1) as determined by reverse transcription polymerase chain reaction (RT-PCR)/capillary electrophoresis with laser-induced fluorescence detection (CE/LIF) for ad libitum and restricted broiler breeders at three stages of production. Values are means ± SEM, $n = 5$. Means without a common letter are significantly different, $P < 0.05$; n.d. designates expression levels below the limits of detection.

ACC and SCD1. SREBP-1 gene expression was positively correlated with all of the lipogenic enzyme genes studied. This undoubtedly reflects the role of this key transcription factor in coordinating hepatic lipogenesis. In addition, adipose tissue LPL expression was positively correlated with all of the major lipogenic enzyme genes, possibly indicating a functional coordination in the expression of this gene affecting lipid deposition in adipose tissue with those genes regulating hepatic lipid production.

Significant ($P < 0.05$) correlations were also identified among circulating levels of key metabolic hormones and the level of expression of the genes involved in lipogenesis (**Table 6**). Insulin and T_3 were positively correlated with hepatic lipogenic genes, whereas glucagon exhibited a negative correlation with each of these genes.

Circulating 17β-estradiol levels were significantly ($P < 0.05$) correlated with the level of expression of genes involved in lipid transport (**Table 7**). FABP, apoVLDL-II and apoB were positively correlated with 17β-estradiol, whereas apoA1 was negatively correlated. Significant ($P < 0.05$) negative correlations were also found between the level of expression of the ApoA1 gene and those for FABP, apoVLDL-II and apoB. Expression levels of FABP, apoVLDL-II and apoB were positively correlated with each other, perhaps indicating a functional interrelationship of these genes in hepatic lipid transfer, especially during egg production.

An excellent linear relationship ($y = 0.087323x - 0.259947$, $r^2 = 0.9999$) between hepatic cytosolic ME enzymatic activity

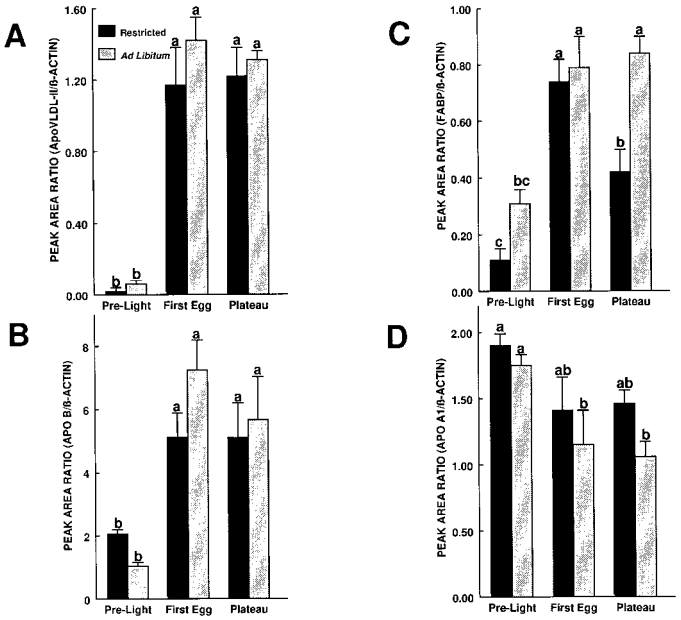

FIGURE 3 Hepatic expression (relative to β-actin) of the genes coding for (*A*) apoVLDL-II, (*B*) apoB, (*C*) fatty acid binding protein (FABP) and (*D*) apoA1 as determined by reverse transcription polymerase chain reaction (RT-PCR)/capillary electrophoresis with laser-induced fluorescence detection (CE/LIF) for ad libitum and restricted broiler breeders at three stages of production. Values are means ± SEM, $n = 5$. Means without a common letter are significantly different ($P < 0.05$).

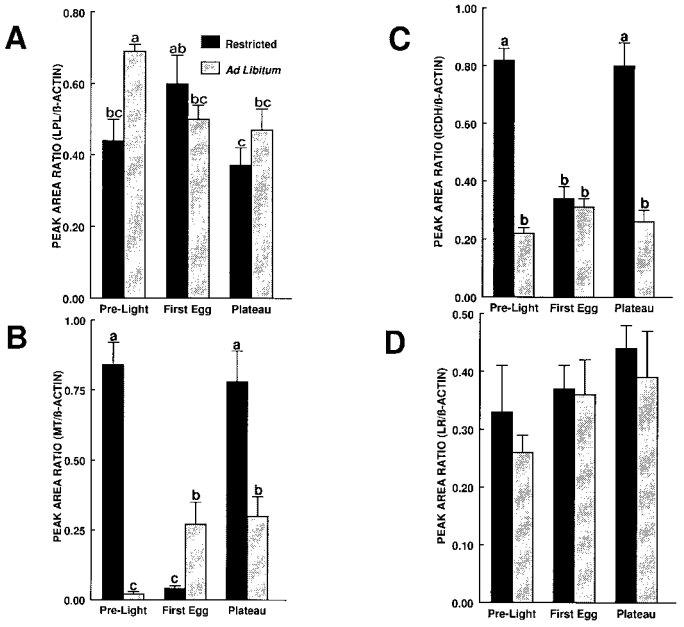

FIGURE 4 Expression (relative to β-actin) of the genes coding for (*A*) adipose tissue lipoprotein lipase (LPL), (*B*) hepatic metallothionein (MT), (*C*) hepatic cytosolic isocitrate dehydrogenase (ICDH) and (*D*) hepatic leptin receptor (LR) as determined by reverse transcription polymerase chain reaction (RT-PCR)/capillary electrophoresis with laser-induced fluorescence detection (CE/LIF) for ad libitum and restricted broiler breeders at three stages of production. Values are means ± SEM, $n = 5$. Means without a common letter are significantly different ($P < 0.05$).

and expression of the ME gene was observed for the ad libitum birds at each of the sampling periods and for the restricted birds before photostimulation (**Fig. 5**). However, this relationship did not hold true for the restricted birds during egg production (first egg and plateau phases), suggesting the existence of additional post-transcriptional and/or post-translational mechanisms (e.g., mRNA turnover or allosteric control) for regulating enzyme activity level within the livers of these birds during this period. In contrast, changes in ME activity in the ad libitum group of birds appeared to be regulated exclusively at the level of gene transcription.

DISCUSSION

This study compared the effects of feed restriction on the expression of selected lipogenic genes in broiler breeders during the pullet-to-breeder transition period. Unrestricted feeding of breeders during egg production led to increased body and abdominal fat pad weights and lowered egg production efficiency compared with birds subjected to restricted feeding. It has been suggested that excessive body weight gain, resulting from overfeeding of female birds during the breeding phase of production, accelerates ovarian follicular maturation such that more ovulations occur than the oviduct can effectively process (2). This leads to an increase in the production of defective or nonsettable eggs. The higher incidence of abnormal (nonsettable) eggs produced by the ad libitum group in this study is consistent with that suggestion. Because the average chicken egg yolk contains ~4 g of triglycerides (21), sustained egg production is an energy-intensive process, requiring a large increase in the supply of lipid (triglycerides) to support the demands of new yolk formation. This increased supply of lipid can originate either from hepatic lipogenesis or from intestinal absorption of dietary lipid. Because typical poultry rations are relatively low in fat (<10 g/100 g feed), the liver synthesizes a major portion of lipid destined for deposit in adipose tissue and the ovary of the laying hen (9). In breeder hens, excessive accumulation of lipid in adipose tissue reduces feed efficiency and diverts necessary energy supply away from egg production.

Nutritional (energy) status and the subsequent responses of key plasma metabolic hormones (insulin, glucagon and T_3) are important factors that determine the level of hepatic lipogenesis in birds (11). Lipogenesis is dependent on glucose metabolism to provide the acetyl CoA necessary to initiate and sustain de novo fatty acid synthesis (see Fig. 1). Carbohydrate (glucose) availability, and thus lipogenic activity, would be expected to be higher in ad libitum compared with restricted birds. This was in fact the case as indicated by significantly ($P < 0.05$) higher expression of hepatic SREBP-1, ME, ACL, ACC, FAS and SCD1 genes in ad libitum compared with restricted birds just before photostimulation. Feed restriction also significantly ($P < 0.05$) affected circulating levels of insulin, glucagon and T_3. Both insulin and T_3 induce a number of genes coding for lipogenic enzymes including ME, ACC and FAS, as well as the key transcription factor that coordi-

TABLE 5

Correlations between the expression of genes related to lipogenesis and lipid uptake in broiler breeder chickens[1]

Gene	SREBP1	ACL	ME	ACC	FAS	SCD1	LPL
				r(*P*)			
SREBP-1	1.00000	0.75407 (<0.0001)	0.72363 (<0.0001)	0.72369 (<0.0001)	0.78982 (<0.0001)	0.68441 (<0.0001)	0.59989 (0.0005)
ACL		1.00000	0.77979 (<0.0001)	0.65879 (<0.0001)	0.77906 (<0.0001)	0.60956 (<0.0001)	0.61871 (0.0003)
ME			1.00000	0.67893 (<0.0001)	0.80915 (<0.0001)	0.73780 (<0.0001)	0.65201 (<0.0001)
ACC				1.00000	0.92037 (<0.0001)	0.83892 (<0.0001)	0.65744 (<0.0001)
FAS					1.00000	0.89462 (<0.0001)	0.67176 (<0.0001)
SCD1						1.00000	0.56850 (0.0010)
LPL							1.00000

[1] Pearson's correlation coefficients (*r*) are shown with *P* values in parentheses.

TABLE 6

Correlations between plasma hormone levels and the expression of genes related to lipogenesis in broiler breeder chickens[1]

Hormone	SREBP-1	ACL	ME	ACC	FAS	SCD1
			r(*P*)			
Insulin	0.63970 (0.0001)	0.61762 (0.0003)	0.63342 (0.0002)	0.60799 (0.0004)	0.64792 (0.0001)	0.47303 (0.0083)
Glucagon	−0.67693 (<0.0001)	−0.57514 (0.0009)	−0.60386 (0.0004)	−0.94008 (<0.0001)	−0.87159 (<0.0001)	−0.78326 (<0.0001)
T_3	0.60795 (0.0004)	0.74491 (<0.0001)	0.64136 (0.0001)	0.61914 (0.0003)	0.72808 (<0.0001)	0.49519 (0.0054)

[1] Pearson's correlation coefficients (*r*) shown with *P* values in parentheses.

nates the majority of lipogenic enzyme gene expression, SREBP-1 (11,13,22). Glucagon, on the other hand, specifically inhibits the expression of the SREBP-1 gene and lipogenic enzyme genes such as ACC and SCD1 (13,23,24). The surge in lipogenic gene expression in ad libitum birds before photostimulation undoubtedly reflects increased feed consumption occurring in this group after the earlier period of feed restriction (up to 21 wk of age), and it tended to diminish as the birds came into egg production. This surge was accompanied by elevated plasma levels of insulin and T_3 and reduced glucagon levels in ad libitum birds compared with those maintained on restricted feeding. These responses are analogous to what occurs in response to starvation followed by refeeding, which provides additional glucose to the liver and alters circulating levels of insulin, glucagon and T_3 to enhance lipogenic activity (11). In the restricted group, the up-regulation of lipogenic genes was delayed until after photostimulation (first egg). This may have been the result of an increased allotment of feed offered to the birds during this time (see Table 1) or the effect of increased circulating estrogen level in response to photostimulation, which enhances hepatic lipogenic activity in laying hens to meet the demands for egg yolk formation (9). Specific changes in plasma hormones (i.e., increased insulin and T_3 with lowered glucagon) produced a physiologic state, promoting lipogenesis in the restricted birds at the time of first egg compared with the prelight period.

At the plateau phase of egg production, expression levels of ACC, FAS, SCD1 and FABP genes were significantly ($P < 0.05$) higher in the livers of ad libitum birds compared with their restricted counterparts. Because the major mode of regulation of hepatic lipogenesis by nutritional status is at the level of gene transcription (11), this could signal a higher rate of lipogenesis in the ad libitum birds at this time. It could also have contributed to the increased abdominal fat pad size observed in ad libitum compared with restricted birds. It is interesting to note that the reaction catalyzed by ACC constitutes the rate-limiting step in the fatty acid synthetic pathway (25) and that, in our study, each of the genes significantly ($P < 0.05$) up-regulated by unrestricted feeding was at or below the ACC step in this pathway (see Fig. 1). Perhaps coordinate expression of these functionally interrelated genes was established in response to the level of feeding. An indication of this might be found in the high degree of correlation among expression levels of these hepatic lipogenic genes (see Table 5). Hillgartner et al. (25) previously reported that dietary control of FAS and ME gene transcription is coordinated with that of ACC in chicken liver, and they further suggested that common control mechanisms are involved in the nutritional regulation of hepatic lipogenic enzyme genes in chickens. The coordination of lipogenic gene transcription in response to nutritional status appears to be tissue specific and most likely involves unique *cis*-acting sequences and *trans*-acting factors present in the liver (25).

Because mRNA levels do not always correlate directly with the amount of functional protein produced within cells, determining the functional relationship between gene expression measurements (i.e., mRNA levels) and enzyme activity is one way to gauge the relevance of the expression data to actual physiologic/biochemical effects. We provided one example of this, showing a direct linear relationship between hepatic gene expression values and measurements of cytosolic enzyme activity for ME (see Fig. 5). In ad libitum birds, this direct relationship held at each of the three phases of production monitored. However, it did not apply to restricted birds during

TABLE 7

Correlations between plasma 17β-estradiol levels and the expression of genes related to lipid transport in broiler breeder chickens[1]

Variable	Estradiol	ApoVLDL-II	ApoB	FABP	ApoA1
			r(*P*)		
Estradiol	1.00000	0.50354 (0.0046)	0.62742 (0.0002)	0.57366 (0.0011)	−0.42952 (0.0178)
ApoVLDL-II		1.00000	0.83304 (<0.0001)	0.78894 (<0.0001)	−0.71713 (<0.0001)
ApoB			1.00000	0.79267 (<0.0001)	−0.75772 (<0.0001)
FABP				1.00000	−0.87363 (<0.0001)
ApoA1					1.00000

[1] Pearson's correlation coefficients (*r*) are shown with *P* values in parentheses.

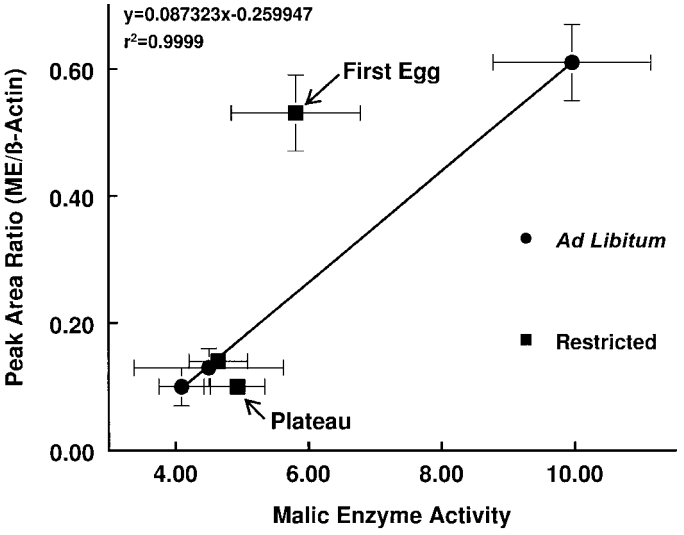

FIGURE 5 Expression ratio (relative to β-actin) of hepatic cytosolic malic enzyme (ME) plotted as a function of the enzyme activity for ad libitum and restricted broiler breeders at three stages of production. Linear regression analysis (excluding the restricted groups at first egg and at plateau) was used to characterize the relationship between enzyme activity and gene expression. The equation used to plot the line and the correlation coefficient are shown on the figure. Values are means ± SEM, n = 5 determinations each of gene expression and enzymatic activity.

egg production (first egg and plateau). This suggested the involvement of other undefined factors/processes regulating the level of ME gene transcription and/or enzyme activity. Short-term adaptive changes in ACC enzyme activity can be achieved by covalent modification of the protein (i.e., phosphorylation) and allosteric control (by citrate) in addition to long-term transcriptional regulation mediated by insulin, glucose, T_3 and glucagon (11,23,25). Similar short-term mechanisms have not been reported previously for the regulation of ME activity (11,26). Although the discrepancies between ME expression and enzyme activity data in the restricted group at first egg and plateau production phases of this study remain unexplained, they may indicate additional post-transcriptional regulation, perhaps involving mRNA stability or translational efficiency.

Two cytosolic, NADP(+)-dependent hepatic enzymes (ME and ICDH) that provide reducing equivalents in the form of NADPH were investigated in this study. Malic enzyme has previously been suggested to provide the majority of reducing equivalents required by FAS for fatty acid biosynthesis in the liver of birds (11). On the basis of the differential patterns of gene expression observed for ME and ICDH, one could speculate that ICDH does not play a similar role. Instead, ICDH may perform a completely different function, such as supplying NADPH for the regeneration of glutathione and other systems involved with intracellular defense against oxidative damage (27,28). Similarly, MT has also been suggested to play a role in energy metabolism by acting as an intracellular antioxidant (29,30). It is important to point out that the pattern of gene expression observed for both MT and ICDH in this study was quite similar (see Fig. 4). Also, the expression of both genes was highly correlated with circulating levels of glucagon, suggesting a role for glucagon in the regulation of these two genes. Elevated plasma glucagon is indicative of the presence of stress accompanied by reduced lipogenesis, increased lipolysis and increased potential for the generation of reactive oxygen species as by-products of fatty acid oxidation. However, the actual role(s) that ICDH and/or MT might play, if any, in the maintenance of cellular redox state and energy homeostasis during stress in broiler breeders remains to be elucidated.

In birds, the accumulation of lipid in extrahepatic tissues results to a large extent from the combined effects of hepatic lipogenesis and lipoprotein production. Therefore, plasma triglyceride levels are dependent on the level of lipogenesis that takes place in the liver and on the action of systems involved in the packaging and export of triglycerides in the form of VLDL particles (Fig. 1). In birds, specific mechanisms exist to partition energy between the ovary (developing yolk) and adipose tissue stores during periods of active egg production (9). With the onset of egg production, a shift in the type of VLDL produced by the liver occurs. In response to increased circulating levels of estrogen, the liver redirects the VLDL assembly process toward the production of a new and smaller subclass of lipoprotein particles (31). This new VLDL particle, designated "$VLDL_y$" for yolk-targeted, contains large amounts of apoVLDL-II in addition to apoB. The presence of apoVLDL-II appears to specifically inhibit the action of LPL, thus making $VLDL_y$ unavailable to tissues via LPL hydrolysis. Instead, the developing yolk follicle, via a receptor-mediated process that specifically recognizes the apoB component, assimilates the intact $VLDL_y$ complex (21,31). The transfer of triglycerides from plasma VLDL into adipose and other tissues such as skeletal and cardiac muscle involves the action of LPL, which hydrolyzes triglycerides to fatty acids and glycerol (9). The fatty acids are then taken up and reesterified to form new triglyceride deposits. Although it has been suggested that adipose tissue LPL is somewhat resistant to nutritional or hormonal changes, little is known about LPL gene regulation in birds (9,32). The fact that abdominal fat pad size increased in both groups of birds in this study during periods of active egg production indicates that lipid continues to be deposited into adipose tissue in addition to yolk. Moreover, it has been reported that overfeeding laying hens can negatively affect egg production, alter plasma lipoprotein profiles and, in extreme cases, lead to an excessive accumulation of lipid in the liver, giving rise to fatty liver hemorrhagic syndrome (33,34).

In conclusion, a better understanding of the mechanisms governing the partitioning of lipid stores between adipose tissue and ovarian follicles (yolk) is required to develop strategies to effectively control energy metabolism in female broiler breeders. Moreover, studying changes in lipogenic gene expression in response to restricted vs. ad libitum feed intake should provide useful information for evaluating energetic efficiency after changes in the feeding regimen. The effect of such events on the partitioning of energy stores among different extrahepatic tissues in broiler breeders warrants further study.

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